

Claims

1. A DNA construction for regulating the expression of a virus structural protein gene by using a recombinase and its recognition sequence wherein a promoter, the recombinase recognition sequence, a drug resistance gene, a polyA addition signal, the recombinase recognition sequence, the virus structural protein gene and a polyA addition signal are arranged in this order.

2. A DNA construction for regulating the expression of a foreign gene by using a recombinase and its recognition sequence wherein the LTR of a retrovirus genome and a packaging signal are followed by the recombinase recognition sequence, a drug resistance gene, a polyA addition signal, the recombinase recognition sequence, the foreign gene and LTR arranged in this order.

3. The DNA construction as set forth in Claim 1, wherein the promoter is CAG.

4. The DNA construction as set forth in Claim 1 or 2, wherein the recombinase and its recognition sequence is Cre recombinase and an loxP sequence.

5. The DNA construction as set forth in Claim 1 or 2, wherein the drug resistance gene is a neomycin resistance gene, a puromycin resistance gene or a hygromycin resistance gene.

6. The DNA construction as set forth in Claim 1 or 2, wherein the drug resistance gene is a low-efficient drug

resistance gene or a short-lived transcript drug resistance gene having a base sequence of a short-lived mRNA of a drug resistance gene.

7. The DNA construction as set forth in Claim 6, wherein the low-efficient drug resistance gene or short-lived transcript drug resistance gene is one originating in a neomycin resistance gene, a puromycin resistance gene or a hygromycin resistance gene.

8. A short-lived transcript drug resistance gene characterized by having a base sequence of a short-lived mRNA of a neomycin resistance gene, a puromycin resistance gene or a hygromycin resistance gene.

9. The short-lived transcript drug resistance gene as set forth in Claim 8, wherein the mRNA has been made short-lived by using an mRNA unstabilizing signal originating in c-fos.

10. The DNA construction as set forth in Claim 1 or 2, wherein the polyA addition signal is one originating in SV40 or β -globin.

11. The DNA construction as set forth in Claim 1, wherein the retrovirus structural protein gene is a DNA encoding vesicular stomatitis virus (VSV) G protein (VSV-G).

12. The DNA construction as set forth in Claim 2, wherein the retrovirus genome is one originating in Moloney murine leukemia virus (MoMLV).

13. The DNA construction as set forth in Claim 2, wherein

the retrovirus genome is one originating in a lentivirus.

14. The DNA construction as set forth in Claim 2, wherein the foreign gene is a gene to be transferred into cells for gene therapy.

15. The DNA construction as set forth in Claim 14, wherein the gene to be transferred into cells is a gene of a cytotoxic protein.

16. The DNA construction as set forth in Claim 1 for regulating the expression of a virus structural protein by using a recombinase and its recognition sequence wherein a CAG promoter, an loxP sequence, a drug resistance gene, a polyA addition signal, an loxP sequence, a VSV-G gene and a polyA addition signal are arranged in this order.

17. The DNA construction as set forth in Claim 2 for regulating the expression of a foreign gene by using a recombinase and its recognition sequence wherein the LTR of a retrovirus genome and a packaging signal are followed by an loxP sequence, a drug resistance gene, a polyA addition signal, an loxP sequence, the foreign gene and LTR arranged in this order.

18. A prepackaging cell for producing a retrovirus vector wherein the DNA construction as set forth in Claim 1 has been transferred into a retrovirus gag-pol-producing cell.

19. A prepackaging cell containing a virus genome for producing a retrovirus vector wherein the DNA construction as set forth in Claim 2 has been transferred into a retrovirus gag-pol-env-producing cell.

20. The prepackaging cell as set forth in Claim 19 for producing a retrovirus vector wherein the retrovirus envelope protein (env) is one originating in an ecotropic or amphotropic murine leukemia virus.

21. A prepackaging cell containing a virus vector DNA for producing a retrovirus vector wherein DNA constructions as claimed in Claims 1 and 2 have been transferred into a retrovirus gag-pol-producing cell.

22. The prepackaging cell as set forth in any of Claims 18, 19, 20 and 21 for producing a retrovirus vector wherein the retrovirus is murine leukemia virus (MLV).

23. The prepackaging cell as claimed in any of Claims 18, 19, 20 and 21 for producing a retrovirus vector wherein the retrovirus is originated from a lentivirus.

24. A process for preparing a retrovirus vector for gene therapy which comprises transferring a DNA with the recombinase expression into a prepackaging cell containing a virus genome as claimed in Claim 19 or 21.

25. A retrovirus vector for gene therapy prepared by the process as claimed in Claim 24.

26. A process for preparing a retrovirus vector for gene therapy which comprises transferring into retrovirus gag-pol-producing cells a DNA construction wherein a promoter, a recombinase recognition sequence, a drug resistance gene, a polyA addition signal, a recombinase recognition sequence, a virus structural protein gene and a polyA addition signal are

arranged in this order and another DNA construction wherein the LTR of a retrovirus genome and a packaging signal are followed by a recombinase recognition sequence, a drug resistance gene, a polyA addition signal, a recombinase recognition sequence, a foreign gene and LTR arranged in this order, and then further transferring a DNA with the recombinase expression thereinto.

27. A process for preparing a retrovirus vector for gene therapy which comprises transferring into retrovirus gag-pol-env-producing cells a DNA construction wherein the LTR of a retrovirus genome and a packaging signal are followed by a recombinase recognition sequence, a drug resistance gene, a polyA addition signal, a recombinase recognition sequence, a foreign gene and LTR arranged in this order, and then further transferring a DNA with the recombinase expression thereinto.

28. A process for preparing a retrovirus vector for gene therapy which comprises transferring into retrovirus gag-pol-producing cells containing a retrovirus genome encoding a foreign gene a DNA construction wherein a promoter, a recombinase recognition sequence, a drug resistance gene, a polyA addition signal, a recombinase recognition sequence, a virus structural protein gene and a polyA addition signal are arranged in this order, and then further transferring a DNA with the recombinase expression thereinto.

29. The process for preparing a retrovirus vector as set forth in any of Claims 24, 26, 27 and 28 whereby a pseudotyped retrovirus is prepared, characterized in that a negatively

charged, high-molecular weight substance is contained in the liquid culture medium.

30. A process for preparing a retrovirus vector whereby a pseudotyped retrovirus is prepared, characterized in that a negatively charged, high-molecular weight substance is contained in the liquid culture medium.

31. The process for preparing a retrovirus vector as set forth in Claim 29 or 30, wherein the negatively charged, high-molecular weight substance is one selected from among heparin, heparan sulfate and chondroitin sulfate.

32. The process for preparing a retrovirus vector as set forth in Claim 29 or 30, wherein the pseudotyped retrovirus is Moloney murine leukemia virus.

33. The process for preparing a retrovirus vector as set forth in Claim 29 or 30, wherein the pseudotyped retrovirus is originated from a lentivirus.